PhenoSense HIV Entry Assay

Envelope Expression Vector: pHIVenv
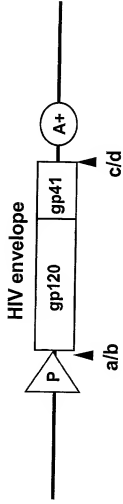
HIV-1 Expression Vector: pHIVlucΔU3
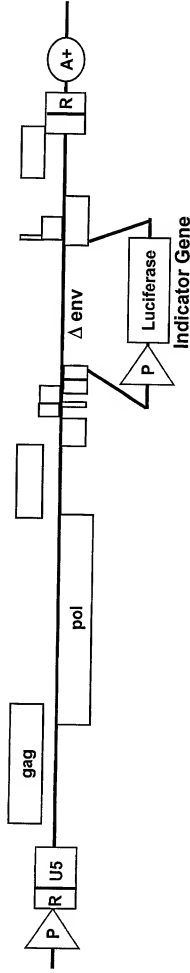

Fig. 1A

PhenoSense™ HIV: Cell Assay

Transfection

pHIVlucΔU3 DNA

pHIVenv DNA

+

Infection

virus entry inhibitors added

CD4 +
CCR5 +
CXCR4 +

Fig. 1B

HIV Envelope Expression Strategies

Fig. 2

2052260" 7204/2007
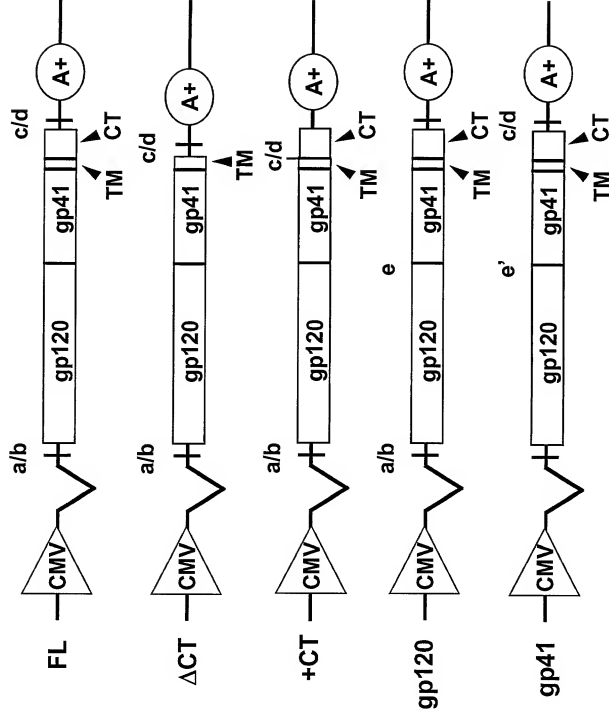
Co-Receptor Tropism Screen
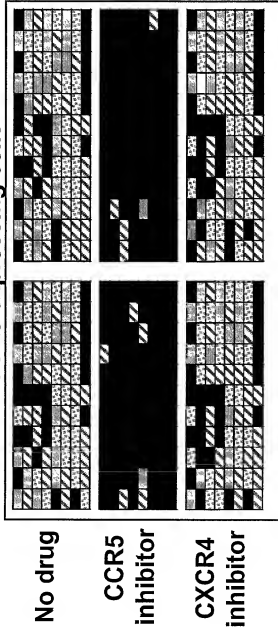
Fig. 3A

Legend:
- ■ <100 RLU
- ▦ 100-1000 RLU
- ▨ 1000-10,000 RLU
- ▩ >10,000 RLU

CCR5-expressing cells
- No drug
- CCR5 inhibitor
- CXCR4 inhibitor

CXCR4-expressing cells
- No drug
- CCR5 inhibitor
- CXCR4 inhibitor

Replicate 1   Replicate 2
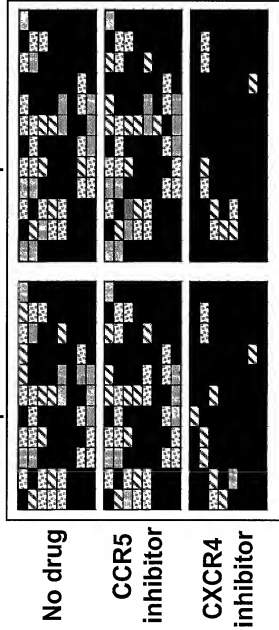
Co-Receptor Tropism Assay Interpretation

	CXCR4
	CCR5
	DUAL or MIXED
	NON-VIABLE

active RLU limit: 100
tropism ratio limit: 5

21

	R5	X4	R5:X4
no drug	67,389	72	936
R5 inhibitor	99		
X4 inhibitor	42,295		
%inhib by R5 inhibitor	100		
%inhib by X4 inhibitor	37		

95

	R5	X4	R5:X4
no drug	43	42	
R5 inhibitor			
X4 inhibitor			
%inhib by R5 inhibitor			
%inhib by X4 inhibitor			

3

	R5 cells	X4 cells	R5:X4
no drug	34	26,471	0
R5 inhibitor		19,258	
X4 inhibitor		32	
%inhib by R5 inhibitor		27	
%inhib by X4 inhibitor		100	

76

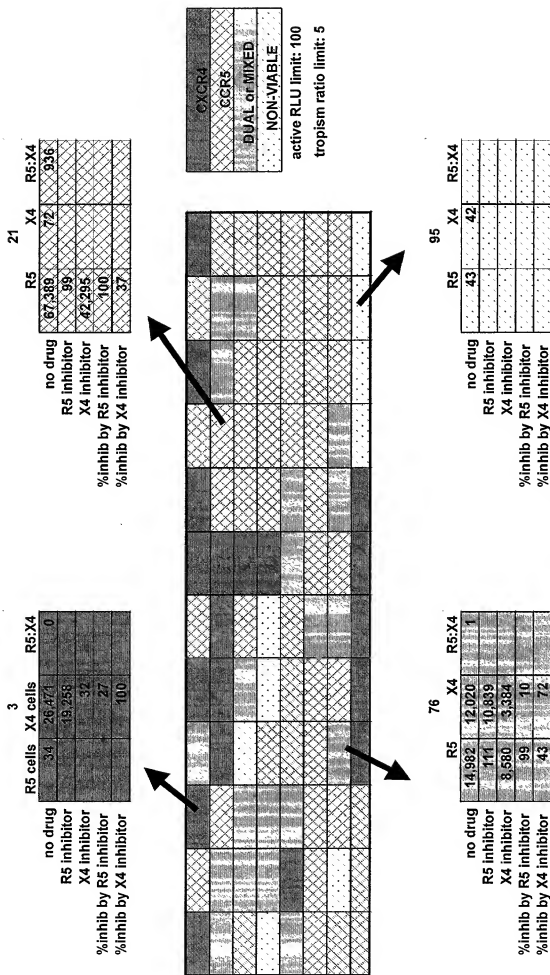|	R5	X4	R5:X4
no drug	14,982	12,020	1
R5 inhibitor	111	10,839	
X4 inhibitor	8,580	3,384	
%inhib by R5 inhibitor	99	10	
%inhib by X4 inhibitor	43	72	

Fig. 3B

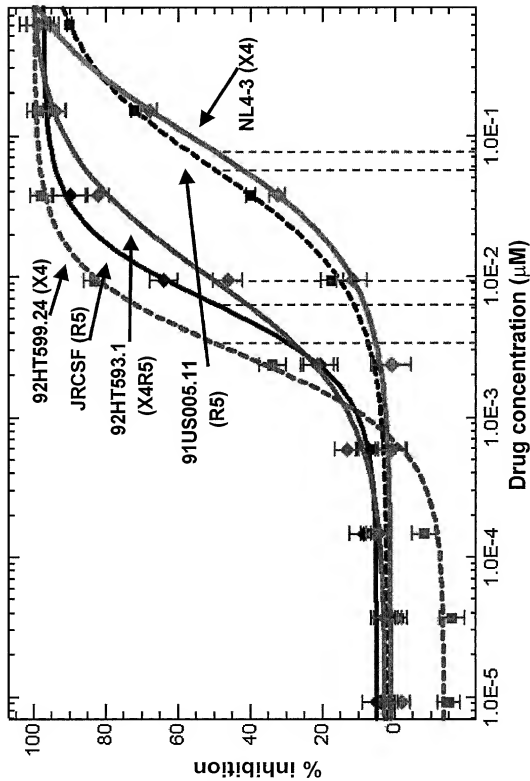# Entry Inhibitor Susceptibility: Fusion Inhibitor

Fig. 4A

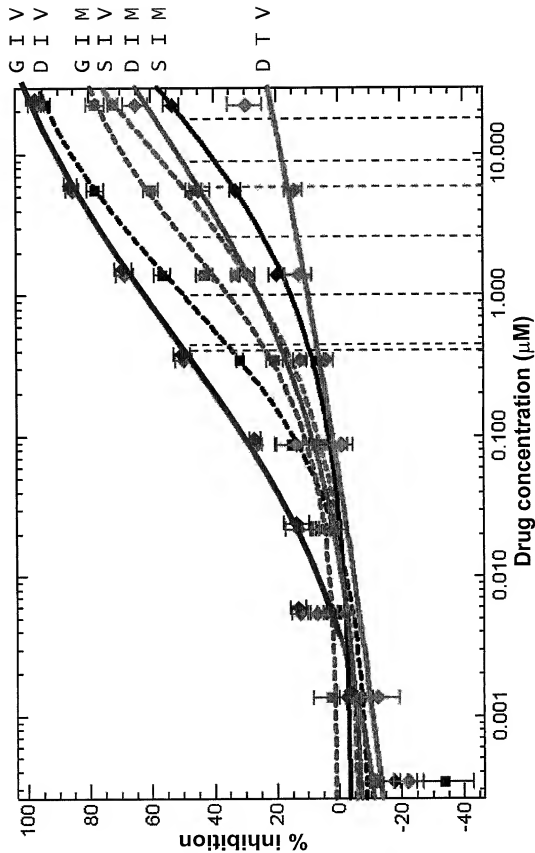# Reduced Susceptibility: Fusion Inhibitor

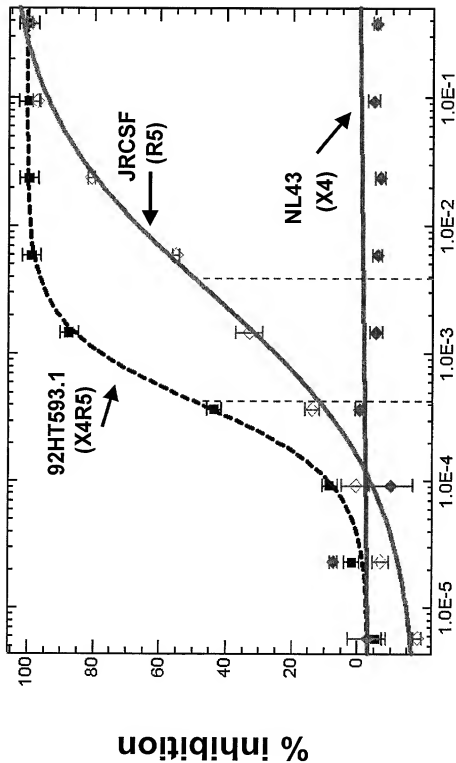Fig. 4B

Entry Inhibitor Susceptibility: CCR5 Inhibitor

Fig. 5A

Drug: R5 Inhibitor
Cell: CD4/CCR5

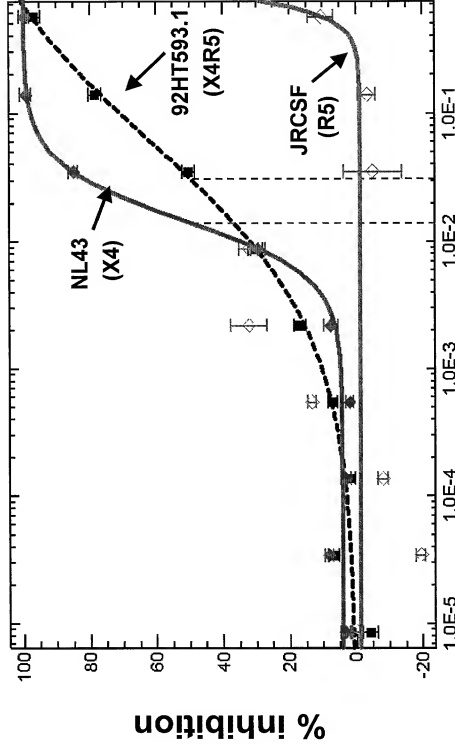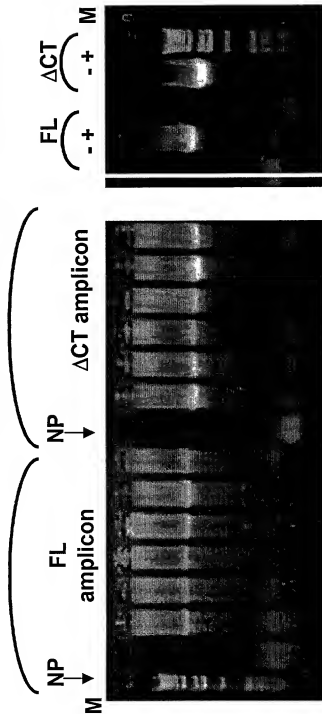# Entry Inhibitor Susceptibility: CXCR4 Inhibitor

Fig. 5B

Drug: X4 Inhibitor
Cell: CD4/CXCR4

Envelope Sequence Amplification

	FL amplicon						ΔCT amplicon					
M	1	2	3	4	5	6	1	2	3	4	5	6

Co-Receptor Tropism

	1 2 3 4 5 6
X4	
R5	
X4/R5	
Undefined	

	# of isolates
	15
	24
	15
	35

Envelope Subtype

	# of isolate
Clade A	2
Clade B	76
Clade C	7
Clade D	1
Clade E	3

1. R5
2. R5/X4
3. R5
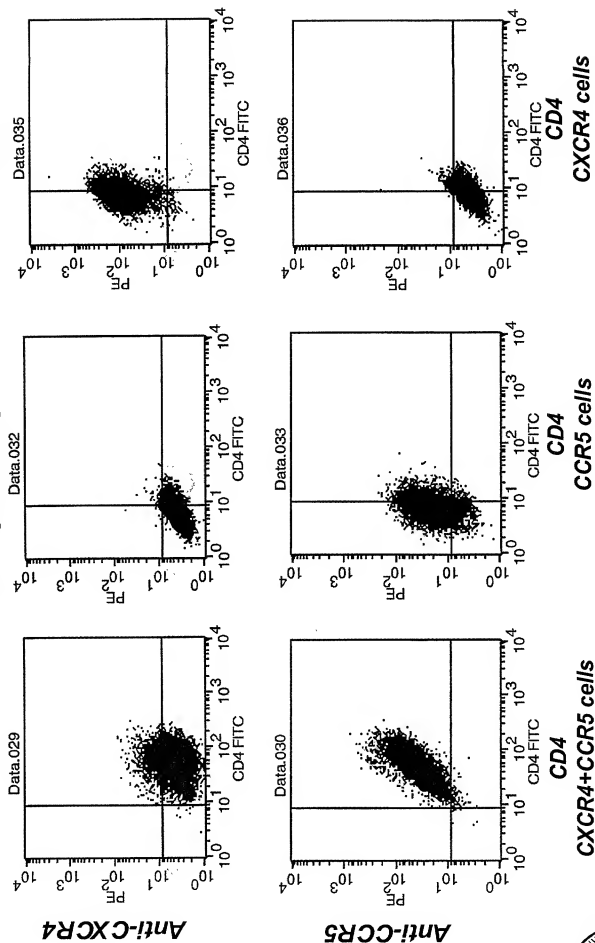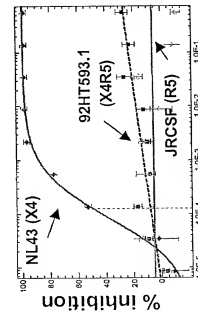4. R5/X4
5. R5
6. R5/X4
NP: HIV negative plasma

Fig. 6

Fig. 7

Target Cell Receptor and Co-Receptor Expression

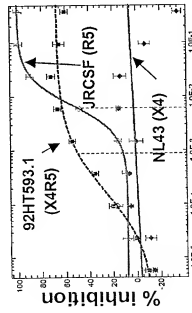## Inhibition By Co-Receptor Antagonists
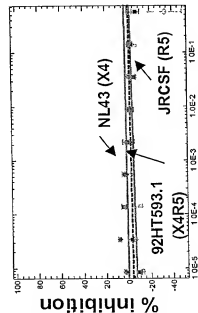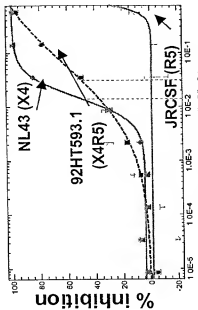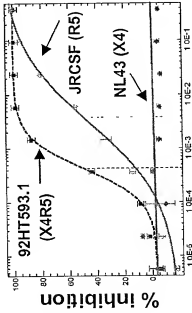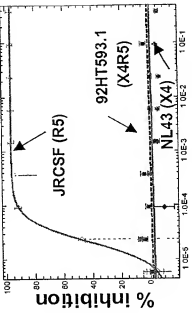
Fig. 8

Fusion Inhibitor Peptides

HIV-1 gp41

QARQLLSGIVQQQ

YLKDQQLL

YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF
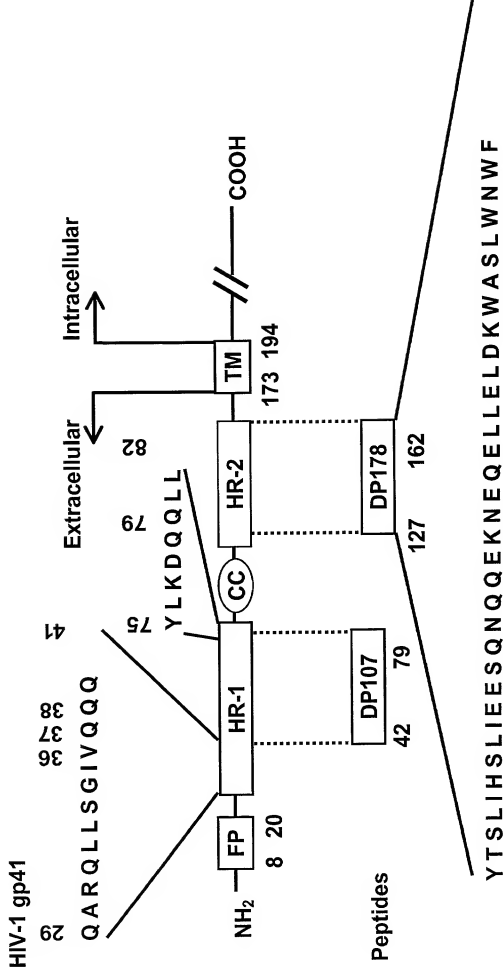
Fig. 9

Rimsky, et al., J. Virol. 72 (2):986-993

Patient Virus v. Patient Antibody

Virus Date	Plasma (Antibody) Date								
	9/2/99	11/29/99	2/29/00	5/31/00	8/30/00	11/22/00	2/14/01	5/30/01	9/11/01
9/2/99	26	219	675	1403	2670	2089	2190	2363	2411
11/29/99	29	179	1024	2151	3733	3152	2808	2953	3086
2/29/00	27	35	78	358	1769	1939	2247	3112	4345
5/31/00	36	67	82	200	795	1078	1371	2208	3375
8/30/00	19	48	36	64	76	166	556	937	1407
11/22/00	29	43	64	76	90	119	374	721	1234
2/14/01	42	65	61	152	117	134	122	289	526
5/30/01	41	66	82	84	85	113	78	107	296
9/11/01	42	62	56	62	85	77	55	61	95

Reference Virus									
NL43	17	138	294	956	1172	953	1584	1868	2143
JRCSL	24	37	35	60	87	97	105	152	209

Fig. 10